Redox regulation of ischemic limb neovascularization - What we have learned from animal studies

Matsui, Reiko; Watanabe, Yosuke; Murdoch, Colin E.

Published in:
Redox Biology

DOI:
10.1016/j.redox.2017.04.040

Publication date:
2017

Redox Biology 12 (2017) 1011–1019

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Review article

Redox regulation of ischemic limb neovascularization – What we have learned from animal studies

Reiko Matsui[a,*], Yosuke Watanabe[b], Colin E. Murdoch[c]

[a] Vascular Biology Section, Whitaker Cardiovascular Institute, Boston University School of Medicine, USA
[b] Department of Internal Medicine II, University of Yamanashi, Faculty of Medicine, Japan
[c] Aston Medical Research Institute, Aston Medical School, UK

ARTICLE INFO

Keywords:
Ischemic limb
Angiogenesis
Oxidants
GSH adducts
Glutaredoxin

ABSTRACT

Mouse hindlimb ischemia has been widely used as a model to study peripheral artery disease. Genetic modulation of the enzymatic source of oxidants or components of the antioxidant system reveal that physiological levels of oxidants are essential to promote the process of arteriogenesis and angiogenesis after femoral artery occlusion, although mice with diabetes or atherosclerosis may have higher deleterious levels of oxidants. Therefore, fine control of oxidants is required to stimulate vascularization in the limb muscle. Oxidants transduce cellular signaling through oxidative modifications of redox sensitive cysteine thiols. Of particular importance, the reversible modification with abundant glutathione, called *S*-glutathionylation (or GSH adducts), is relatively stable and alters protein function including signaling, transcription, and cytoskeletal arrangement. Glutaredoxin-1 (Glrx) is an enzyme which catalyzes reversal of GSH adducts, and does not scavenge oxidants itself. Glrx may control redox signaling under fluctuation of oxidants levels. In ischemic muscle increased GSH adducts through Glrx deletion improves *in vivo* limb revascularization, indicating endogenous Glrx has anti-angiogenic roles. In accordance, Glrx overexpression attenuates VEGF signaling *in vitro* and ischemic vascularization *in vivo*. There are several Glrx targets including HIF-1α which may contribute to inhibition of vascularization by reducing GSH adducts. These animal studies provide a caution that excess antioxidants may be counter-productive for treatment of ischemic limbs, and highlights Glrx as a potential therapeutic target to improve ischemic limb vascularization.

1. Introduction

Use of antioxidants has gained prevalence to protect and improve our health, based on the assertion that excess oxidants are harmful introducing cellular “oxidative stress”. However, clinical studies have failed to prove the efficacy of antioxidant therapy to combat cardiovascular disease [1,2]. In contrast, growing evidence supports the concept that physiological levels of oxidants or free radicals are essential to cellular signaling and to promote ischemic angiogenesis [3]. Vascular endothelial growth factor (VEGF)-induced reactive oxygen species (ROS) is an important component for mitotic signaling [4]. The *in vivo* roles of oxidants and antioxidants have been investigated using genetically modified mice which overexpress or lack specific enzymes involved in controlling redox homeostasis. For example, decrease in oxidants by Nox2 deletion impaired revascularization in ischemic limb study [5,6], while upregulated Nox4 expression improved revascularization in ischemic limbs [7,8], indicating beneficial effects of elevated oxidants. This paradoxical concept is possibly true also in aging. Although oxidative stress has been considered to be a promoting factor for aging process [9], recent studies show that modest elevation of oxidants increases lifespan at least in *C. elegans*. [10].

In this review we use the term “Oxidants” as a general term including reactive oxygen species (ROS, *e.g.* superoxide anion) and reactive nitrogen species (RNS, *e.g.* peroxynitrite). Oxidants control cellular activities including proliferation, migration, apoptosis, and transcription through post-translational modifications of various target proteins. The role of NADPH oxidases (NOX) as a source of oxidants in VEGF signaling and angiogenesis has been well studied and reviewed elsewhere [11,12]. Recently a reversible modification on protein thiols is emerging as a mechanism which generates “redox signaling” [13,14]. This review will highlight how *in vivo* animal studies are shedding light on the role of oxidants and redox signaling in ischemic vascularization, focusing on hindlimb ischemia model. Emphasize will be placed on the importance of oxidative modification with protein glutathione (GSH) adducts, or *S*-glutathionylation, on vascularization.

* Corresponding author.
E-mail address: rmatsui@bu.edu (R. Matsui).

http://dx.doi.org/10.1016/j.redox.2017.04.040
Received 20 February 2017; Received in revised form 8 April 2017; Accepted 24 April 2017
Available online 04 May 2017

2. Animal models of ischemic neovascularization

In vitro assays assessing endothelial or macrophage angiogenic function do not always reflect the *in vivo* outcome [15]. Cell-cell interaction, paracrine factors, and tissue specific components make complex microenvironment to support *in vivo* vessel formation. After tissue is exposed to ischemia, impaired blood flow is recovered by capillary formation (angiogenesis) and artery formation (arteriogenesis). Angiogenesis is defined as the new capillary formation form pre-existing vessels, while arteriogenesis refers to remodeling of larger vessels (arterioles and arteries) which may be formed from pre-existing collateral vessels or anastomosis [16] and also can be developed from new capillaries. Revascularization after artery occlusion is considered as the combined process of arteriogenesis and angiogenesis, and the regulatory mechanisms are still not fully understood [17,18]. Herein we mainly focus on the hindlimb ischemia model, and additionally compare with cardiac ischemia by coronary artery occlusion.

2.1. Role of oxidants in hindlimb ischemia models

Blood flow recovery after unilateral femoral artery ligation in rodents is usually assessed by LASER Doppler imaging [19] as shown in Fig. 1. Capillary density of ischemic muscles, motor function, necrotic area, and micro-computed tomographic imaging or angiography are used to support the finding [18]. Although factors such as animal strain, surgical procedure, and age influence the outcome, the method is a gold standard to study regulatory mechanism of arteriogenesis and angiogenesis following ischemia. The roles of oxidants and antioxidants in ischemic limb revascularization are examined using genetically modified mice, and the major studies are summarized in Table 1.

In an early study elucidated the important role of eNOS-derived nitric oxide (NO). Dietary supplement of L-arginine promoted eNOS activity and improved vascularity in rabbit ischemic limbs. In accordance with that, $eNOS^{-/-}$ mice showed impaired neovascularization. Yet, the expression of vascular endothelial growth factor (VEGF) was unaltered. The impaired vascularization was not improved by administration of VEGF in $eNOS^{-/-}$ mice [20]. Taken together this suggests that eNOS is downstream of VEGF and likely eNOS-derived NO or RNS is required for VEGF signaling.

NADPH oxidases (NOX) are a major source of oxidants in the vasculature. The NOX family of enzymes has differential cell type expression and subcellular localization; in addition, they produce different oxidants. Deletion of NOX2 showed impaired ischemic vascularization [5,6]. Whereas, when NOX4, an enzyme which specifically produces hydrogen peroxide (H_2O_2), is overexpressed in endothelial cells, ischemic vascularization is improved [7,8] which is in accordance with NOX4 improving endothelial function [21]. In contrast, when NOX4 is deleted, ischemic revascularization was impaired [8,22]. In addition, the protective role of oxidants in particular H_2O_2 is supported by endothelial-specific overexpression of catalase which shows impaired vascularization in mouse ischemic limb [23]. Catalase overexpression in myeloid linage cells also showed impaired vascularization associated with decreased macrophages infiltration and inflammatory responses [24]. Deletion of the antioxidant enzyme extracellular superoxide dismutase (ecSOD) causes impaired ischemic vascularization, but the protective role of ecSOD in vascularization may not be due to the lower level of superoxide anion, but rather lack of conversion of superoxide anion to H_2O_2 which is essential to promote VEGF signaling [25,26].

2.2. Role of antioxidants in hindlimb ischemia models

These studies indicate that physiological level of oxidants are required to promote ischemic vascularization. Anti-angiogenic role of endogenous antioxidants is shown in mice which are deficient of nuclear factor erythroid 2-related factor 2 (Nrf 2). Nrf2 is a transcription factor which interacts with antioxidant response element and promotes transcription of genes for antioxidants or detoxifying enzymes. $Nrf2^{-/-}$ mice improved vascularization after hindlimb ischemia in association with decreased hemeoxygenase-1, thioredoxin (Trx)-1, and total GSH level and increase in inflammatory responses [27]. Of interest, another paper [15] showed that *in vitro* angiogenic responses were Nrf2-dependent in endothelial cells and myeloid cells, but $Nrf2^{-/-}$

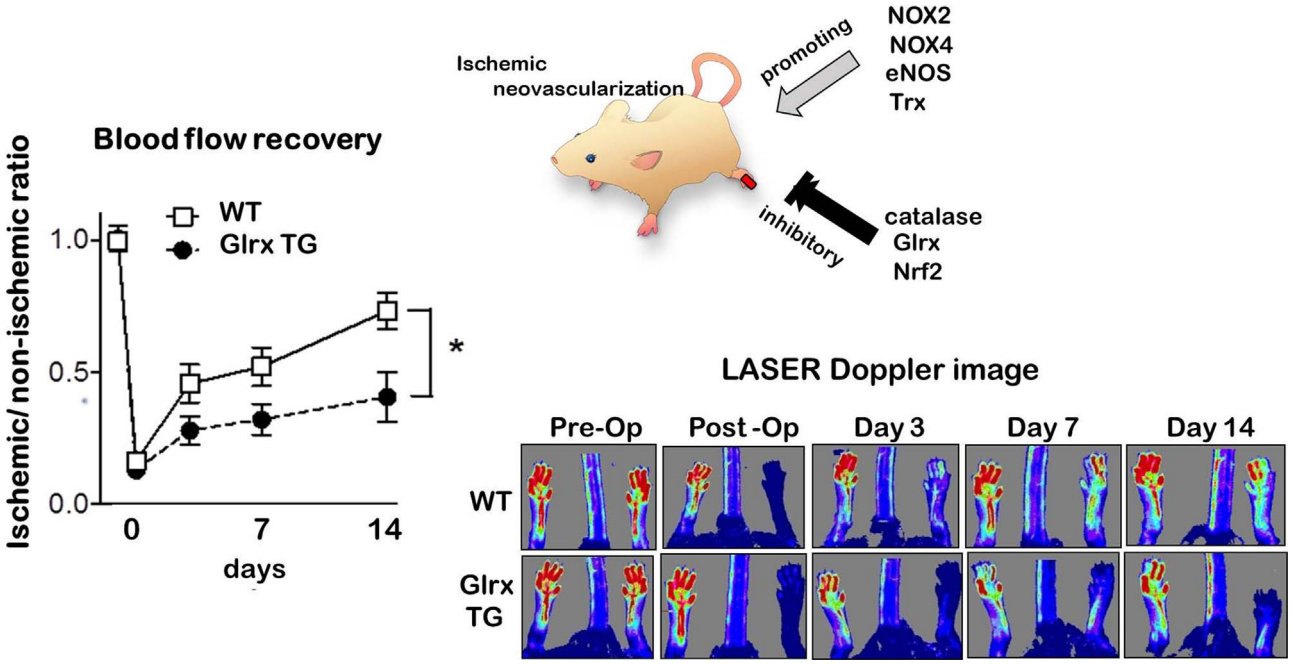

Fig. 1. Hindlimb Ischemia model by femoral artery ligation. After femoral artery ligation, blood flow recovery is assessed by LASER Doppler imaging. An example of images and blood flow ratio (left/right foot) during 2 weeks post-surgery is shown from glutaredoxin (Glrx) transgenic mice study (see Ref. [48]). The upper cartoon indicates that some of oxidants related genes influence ischemic neovascularization. NOXs (NADPH oxidases), eNOS (endothelial nitric oxide synthase), Trx (thioredoxin) are promoting, while catalase, Glrx and Nrf2 are inhibiting ischemic neovascularization. Details are described in the text and references are listed in Table 1.

Table 1
The role of oxidants related enzymes/genes in mouse models of ischemic vascularization.

Gene	Mouse	Disease model	Outcome/ vascularization	Comments	Ref.
NOX2	KO	HLI	Impaired		[5,6]
	KO	HLI after smoke exposure	Protective		[32]
	KO	HLI after HFD	Protective		[33]
	KO	HLI after STZ-diabetes	Protective		[34]
	KO	MI	Protective	Cardiac function	[53]
	EC-TG	MI	No effects		[55]
	CM-TG	MI	Exacerbated	Hypertrophy, fibrosis	[55]
NOX4	EC-TG	HLI	Protective		[7]
	EC-TG	HLI	Protective		[8]
	EC-DN	HLI	Impaired		[8]
	KO	HLI	Impaired		[22]
eNOS	KO	HLI	Impaired		[20]
Catalase	EC-TG	HLI	Impaired		[23]
	Mito-TG	HLI after HFD	Protective	Myopathy less vascularity: NS	[35]
	MCL-TG	HLI	Impaired		[24]
	MCL-TG	MI	Protective	Infarct size smaller	[57]
ecSOD	KO	HLI	Impaired		[25,26]
Trx1	TG	MI	Protective	Cardiac function	[43]
Trx2	EC-TG	HLI	Protective		[41]
Glrx	TG	MI	Protective	Cardiac function	[58]
	TG	HLI	Impaired		[48]
	KO	HLI	Protective		[49]
GCLM	KO	MI (I/R)	Impaired function	Decreased GSH	[29]
	Hetero	HLI	Protective	KO (-/-) no effect	[31]
Nrf2	KO	HLI	Protective	Trx decreased	[27]
	KO	HLI	Protective	Cytokines increased	[15]

Hindlimb and cardiac ischemia studies using genetically modified mice of redox-related genes. NOX: NADPH oxidase, eNOS: endothelial nitric oxide synthase, ecSOD: extracellular superoxide dismutase, Trx: thioredoxin, Glrx: glutaredoxin-1, GCLM: glutamate-cysteine ligase modifier, Nrf2: nuclear factor erythroid 2-related factor 2. KO: global knockout (-/-), Hetero: heterozygous (+/-) mice, TG: global transgenic overexpression, EC-TG: endothelial-specific overexpression, CM-TG: cardiomyocyte-specific overexpression, EC-DN: endothelial-specific dominant negative, Mito-TG: targeted expression to mitochondria, MCL-TG: Myeloid cell linage-specific overexpression, HLI: hindlimb ischemia model, MI: myocardial infarction model, I/R: ischemia-reperfusion, HFD: high fat diet.

mice had improved *in vivo* ischemic vascularization consistent with the former study. This was in association with decreased antioxidant capacity and increased inflammatory responses.

GSH is the most abundant low molecular weight thiol in the cell, consist of glutamate, cysteine, and glycine, and controls cellar redox status by scavenging radicals. Endogenous GSH is produced by ATP-dependent reactions catalyzed by glutamate-cysteine ligase (GCL) and GSH synthase [28]. GSH synthase is transcriptionally regulated by Nrf2, and GCL consists of catalytic (GCLC) and modifier (GCLM) subunits. While deletion of Gclc in mice is lethal, $Gclm^{-/-}$ mice are viable and look normal with decreased GSH in tissues. $Gclm^{-/-}$ mice demonstrated exacerbated cardiac function after ischemia-reperfusion [29] or pressure-overload [30] compared to wild type mice. In hindlimb ischemia studies, $Gclm^{-/-}$ mice did not show different neovascularization from wild type mice. Instead $Gclm^{+/-}$ heterozygous mice showed better blood flow recovery and endothelial cell density [31]. The authors measured superoxide anion levels in ischemic muscles and suggested mild increase in superoxide in $Gclm^{+/-}$ mice stimulated VEGF production and vascularization, but higher superoxide levels in $Gclm^{-/-}$ mice did not. Interestingly, the differences in superoxide levels were at picomolar level. Although total glutathione was decreased, the GSH/GSSG ratio was increased in the ischemic muscle of $Gclm^{+/-}$ mice and decreased of the muscle of $Gclm^{-/-}$ mice [31]. Increases in GSH reductase activity may explain the higher GSH/GSSG ratio [29].

2.3. Hindlimb ischemia in pathological models

Thus, the levels of oxidants are critical to control cellular signaling. Low levels of oxidants in physiological conditions are necessary to promote angiogenesis, but high levels of oxidants in pathological conditions such as diabetes can be harmful. In fact, impaired ischemia-induced vascularization was improved by $NOX2^{-/-}$ mice after smoke exposure [32], high-cholesterol diet [33], or streptozotocin (STZ)-induced diabetes [34]. These data are contrast to the report showing angiogenic role of NOX2 in ischemic limb revascularization [5], and indicate that oxidants derived from NOX2 can inhibit angiogenesis under oxidative stress. Inhibiting NOX2 likely activates endothelial progenitor cells [33,34] and increases VEGF-A levels in diabetic ischemic muscle [34], suggesting production of VEGF-A may be affected in association with oxidative stress. Mitochondria-targeted catalase overexpression was also protective in high-fat fed mice, and decreased necrotic area and improved myopathy and mitochondrial dysfunction despite no significant improvement of limb blood flow recovery. Unlike high-fat fed mice, mitochondrial catalase overexpression did not improve ischemic limb recovery in chow-fed mice [35]. This study indicates that excess mitochondrial oxidants in diet-induced diabetes may be relating to impaired muscle degeneration after ischemia, but its relation to neovascularization is obscure. Increase nitric oxide bioavailability by phosphodiesterase inhibitor can improve ischemic limb vascularization in hypercholesterolemic apolipoprotein $E^{-/-}$ mice [36]. However, excess antioxidants therapy may not be effective as shown in clinical trials because it may affect physiological function of oxidants. Impaired blood flow recovery in atherosclerotic animals was improved by exogenous VEGF [37,38], indicating that VEGF signaling is still functional and augmenting VEGF production is important in the atherosclerotic condition.

2.4. The role of thiol modifying enzymes in hindlimb ischemia

Thioredoxin (Trx) and glutaredoxin (Glrx) are enzymes which reduce protein thiols as part of the cellular antioxidant systems. Trx dominantly reduces intra- or inter- protein disulfide (-S-S-) and Glrx more specifically reduces protein-GSH adducts (R-S-SG). Glrx was originally discovered in *Escherichia coli* lacking Trx [39]. However, Glrx is not just a compensatory enzyme for Trx, but has distinct roles [40]. Overexpression of mitochondrial Trx 2 in endothelial cells promoted vascularization after hindlimb ischemia by increasing NO bioavailability and inhibiting apoptosis signaling kinase-1 (ASK-1) activity [41]. Cytosolic isoform Trx 1 is also protective, promoting endothelial angiogenic activities, and interacting with ASK-1 to inhibit pro-apoptotic pathways [42]. Trx 1 overexpression is protective in mouse myocardial infarction [43] and *ex vivo* ischemia-reperfusion [44]. Thioredoxin-interacting protein (TXNIP) has been considered as an endogenous Trx inhibitor which is increased in diabetes. Interestingly, knockdown TXNIP improved ischemic vascularization in STZ-induced diabetic mice. However, the experiment using a mutant of Trx binding site suggested this anti-angiogenic effects of TXNIP may be independent of Trx activity [45].

There are two isoforms of Glrx in mammalian cells. Glrx1 is localized mainly in the cytoplasm which we refer as Glrx, and Glrx2 is localized in mitochondria or the nucleus depending on splice variants. Human Glrx2 exhibits only 34–36% identity with Glrx1 and its specific activity is less than 10% of Glrx1 [46]. In contrast to Trx which is angiogenic, Glrx inhibits endothelial cell angiogenic properties

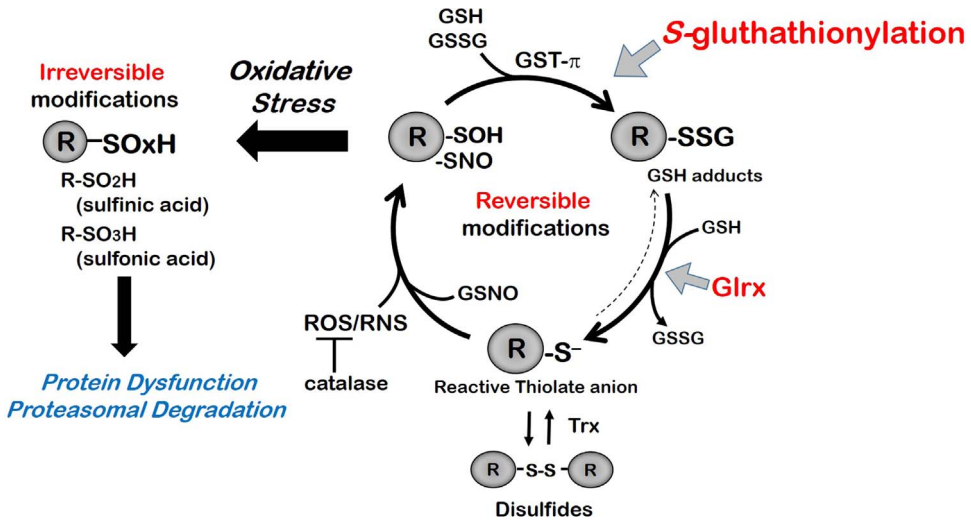

Fig. 2. Redox signaling controlled by protein thiol modifications and glutaredoxin-1 (Glrx). Reactive oxygen and nitrogen species (ROS/RNS) react with thiolate anion (-S^-) and form sulfenic acid (-SOH) or *S*-nitrosylation (-SNO), which can further react with glutathione (GSH) to form *S*-glutathionylation (GSH adducts). Also, ROS/RNS react with GSH forming GSSG or GSNO which may react with thiolate anion, and form reversible modifications. R-SSG formation can be catalyzed by glutathione-s-transferase (GST)-π. Glrx catalyzes reversing GSH adducts to reduced thiol in mono-thiol exchange mechanism in the presence of GSH. Glrx may catalyze formation of GSH adducts in some conditions (dashed arrow). Catalase which scavenges H_2O_2 can inhibit reversible thiol modification including GSH adducts [108]. When oxidants levels increase too much, protein thiols can be irreversibly modified to sulfinic acid (-SO_2H) and sulfonic acid (-SO_3H) which result in protein dysfunction or proteasomal degradation. Thioredoxin (Trx) mainly reduces intra- or inter-protein disulfide.

[47,48]. Glrx overexpressing transgenic (TG) mice impaired [48] and Glrx$^{-/-}$ mice improved ischemic limb vascularization [49]. Glrx controls protein-GSH adducts in the presence of GSH, glutathione reductase, and NADPH, but does not function as an antioxidant *per se* [50]. Glrx$^{-/-}$ mice rather indicated lower oxidants generation after angiotensin II infusion [51]. The role of Glrx on redox signaling and ischemic vascularization will be further discussed later part of this review [52].

2.5. The role of oxidants in cardiac ischemia vs limb ischemia

Cardiac ischemia is studied in animals by ligation of the left anterior descending artery or alternatively transient occlusion followed by reperfusion. The outcome is evaluated by vascularity, apoptosis, infarct area, but mainly assessed by recovery of cardiac function. Inhibiting oxidants seems to be more protective for cardiac ischemia. Global NOX2 deletion is protective in cardiac function with less apoptosis and interstitial fibrosis although cardiac infarct size was similar between NOX2$^{-/-}$ and WT mice [53]. Mice lacking p47^{phox}, cytosolic NOX components, showed similar outcome [54]. This is a clear contrast to the impaired ischemic limb vascularization observed in NOX2 deficient mice [5,6]. Endothelial specific NOX2 overexpression has no effect on cardiac function in myocardial infarction model [55] even though oxidants derived from endothelial NOX2 contribute to cardiac diastolic dysfunction after angiotensin II infusion [56]. In contrast, mice with NOX2 overexpression restricted in cardiomyocytes have enhanced cardiac hypertrophy and fibrosis post myocardial infarction [55]. As mentioned previously, Gclm$^{-/-}$ mice decreased GSH levels and exacerbated cardiac infarction and dysfunction after ischemia reperfusion, while Gclm$^{+/-}$ mice did not show any difference in similar parameters with WT mice [29]. Yet, Gclm$^{+/-}$ mice, but not Gclm$^{-/-}$ mice, promoted vascularization in hindlimb ischemia model [31]. There are number of examples that suggest that redox signaling in response to ischemia seems to be different between heart and skeletal muscle. Myeloid-specific catalase overexpression impaired limb vascularization [24], while the same mice were protected from cardiac infarction [57]. Similarly, Glrx overexpression inhibited limb vascularization [48], while it was protective in myocardial infarction in association with NF-κB activation and inhibited apoptosis [58]. Cardiac tissue must be more sensitive to ischemia as a vital organ compared to limb muscle. In response to acute ischemia, post-ischemic cardiac function is mainly determined by immediate cardiomyocyte apoptosis and mitochondrial function, rather than neovascularization which takes a prolonged amount of time. Whereas the functional outcome after hindlimb ischemia is more directly affected by revascularization potential. In other words, cardiac cell survival is affected by apoptotic signaling in shorter time, while skeletal muscle cell survival is more reliant upon the arteriogenesis and angiogenesis process. Therefore, oxidants-induced angiogenesis signaling may be more critical to the ischemic limb vascularization than cardiac infarction.

3. Molecular mechanism and protein targets

3.1. Redox signaling by thiol modifications

Animal studies reveal that a certain level of endogenous oxidants is essential to promote VEGF signaling and ischemic limb vascularization. Fluctuation of oxidants levels may control cellular signaling through reversible post-translational modification of protein thiols (-SH). Reversible modification on cysteine residues may alter protein function including enzyme activity and protein-protein interaction [13,14,59]. Oxidants (ROS/RNS) react with thiolate anion (-S^-) and form sulfenic acid (-SOH) or *S*-nitrosothiol (-SNO). These relatively unstable intermediates can react with abundant GSH in the cell, result in forming *S*-glutathionylation or GSH protein adducts (R-SSG). Differential identification of *S*-nitrosylation (R-SNO) and *S*-glutathionylation (R-SSG) is not easy since commonly used biotin-switch assay is not specific [60]. Nitrosative stress is shown to induce R-SSG in many proteins, suggesting that R-SNO can be rapidly converted to R-SSG [61–63]. Alternatively, oxidants activate GSH to GSH disulfide (GS-SG), *S*-nitrosoglutathione (GSNO), or other reactive intermediate which react with reduced thiol to form R-SSG [59]. GSH adducts are reversed to reduced thiol in the process of “redox signaling” (Fig. 2). Glrx which catalyzes removal of GSH adducts is a key to control redox signaling. In some condition, Glrx also promotes protein-GSH adducts formation *via* intermediate Glrx-SSG [64,65]. This raises the possibility that Glrx may accelerate redox signaling by promoting dynamics of protein-GSH adducts. Glutathione-S-transferases (GSTs) are potential enzymes which catalyze GSH protein adducts formation. In particular GST-π is indicated to promote GSH protein adducts *in vitro* [66] and in the lung [67]. In contrast, high levels of oxidants in pathological conditions can further oxidize thiolate to sulfinic acid (-SO_2H) and sulfonic acid (-SO_3H) which are irreversible modifications [68], leading to protein

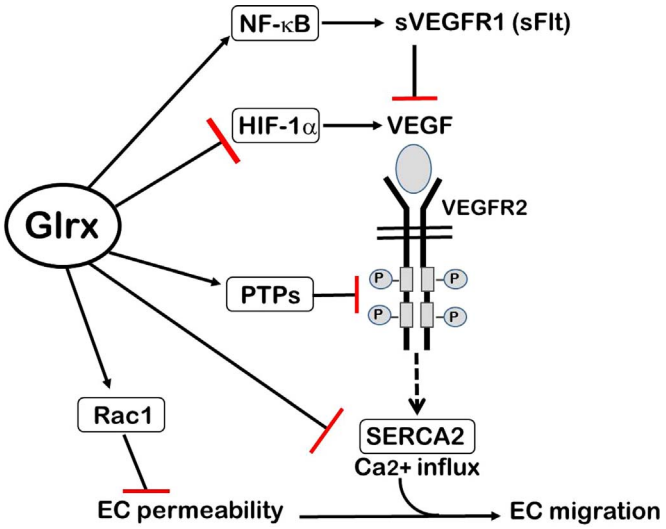

Fig. 3. Glutaredoxin inhibits angiogenic pathway through multiple targets. NF-κB: nuclear factor kappa B, sVEGFR1: soluble VEGF receptor 1 (or sFlt), HIF-1α: hypoxia-inducible factor 1α, PTPs: protein tyrosine phosphatases, SERCA: sarcoplasmic-endoplasmic reticulum calcium ATPase. Arrows indicate activation. Red bars indicate inhibition. Glrx may activates NF-κB, PTP1B or other PTPs, Rac1, while inhibits HIF-1α stability, SERCA2 activity by removing GSH adducts. See the text in details. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

dysfunction and proteasomal degradation (Fig. 2). Some proteins (*e.g.* β-actin) are shown to be *S*-glutathionylated at basal level [69]. Therefore, GSH adducts may be a mechanism to protect redox-sensitive proteins from irreversible oxidation and degradation under fluctuation of oxidants levels. Further studies are required to confirm this concept.

3.2. The targets for GSH adducts in vascularization

A large number of proteins with redox sensitive cysteine(s) have been confirmed to be modified with GSH adducts *in vitro*, but the functional *in vivo* significance has been demonstrated for limited number of proteins [13]. Fewer targets still are known to be reversed and functionally controlled by Glrx *in vivo* (Fig. 3).

3.2.1. VEGF receptor signaling

Downstream of VEGF receptor (VEGFR), protein tyrosine phosphatases (PTP) negatively regulate VEGF signaling [70] and their activity is inhibited by oxidative modification. PTP-1B inhibits phosphorylation of VEGFR2 in endothelial cells [71], and oxidants inactivate PTP-1B *via* GSH adducts on Cys^{215} [72]. Other phosphatases are also involved in VEGFR2 signaling. Low molecular weight (LMW)-PTP (HCPTPA) was found as an interacting protein with the kinase domain of VEGFR2 [73]. LMW-PTP regulates VEGF-induced phosphorylation of focal adhesion kinase and its activity is inhibited by GSH adducts [74]. PTP1B-/- mice demonstrated enhanced *in vivo* angiogenesis and cardiac function after coronary artery ligation [75]. Moreover, EC-specific PTP1B-/- mice also have potentiation of vascularization after femoral artery ligation [76]. Thus, inhibiting PTPs by GSH adducts may be one of targets which oxidants promote during VEGF signaling and vascularization. Another downstream target is calcium uptake by endoplasmic reticulum pump. Sarcoplasmic-endoplasmic reticulum calcium ATPase 2b (SERCA2b) is activated by GSH adducts on Cys^{674} [77]. Overexpression of Cys^{674}Ser mutant SERCA2 or Glrx inhibited VEGF-induced EC calcium influx and migration [47]. Furthermore, heterozygote mice which encode Cys^{674}Ser mutant SERCA2 showed impaired blood flow recovery after hindlimb ischemia and VEGF-induced angiogenic responses were decreased in EC from the mouse [78,79]. Homozygotes of this knock-in mouse impaired fetal development by lack of blood vessel formation, indicating Cys674 of SERCA2 is required for successful fetal angiogenesis [78].

3.2.2. NF-κB signaling

Multiple components of the NF-κB pathway are inhibited by GSH adducts. GSH adducts on inhibitor of nuclear factor kappa-B kinase subunit β (IKKβ) [80], p50 [81], and p65 [48,65] subunits inactivate transcriptional activity, therefore. Therefore, Glrx overexpression enhances NF-κB activation [48,82,83]. Glrx overexpressing TG mice exhibit impaired vascularization with poor motor function recovery in association with increased soluble VEGF receptor 1 (sVEGFR-1 also known as sFlt-1) in muscles and plasma [48]. VEGF binds to VEGFR-1 (fms-like tyrosine kinase-1; Flt) with higher affinity than VEGFR-2, but VEGFR1 transduces a weaker signal than VEGFR-2, and a soluble splice variant (sVEGFR-1) can capture VEGF ligand to prevent its binding to VEGFR2 [84]. Upregulated Glrx induced the anti-angiogenic factor sVEGFR-1 as well as Wnt5a, which may induce sVEGFR-1 expression [85], in EC dependent on NF-κB expression [48]. Interestingly, impaired hindlimb vascularization in diet-induced type 2 diabetic mice was associated with increased sVEGFR-1 in the muscles [86], and Glrx activity was upregulated in diabetic rat tissue [82]. Upregulated Glrx in diabetes may be responsible for sVEGFR-1 induction and poor vascularization. Since Glrx is a NF-κB dependent gene [87], activation of NF-κB in diabetes [88,89] may induce Glrx. Subsequently Glrx-induced NF-κB activation can generate deleterious inflammatory reaction [83]. Inhibition of NF-κB by mutant IκBα, which blocks its degradation and works as a super repressor, increased disorganized vasculature and decreased functional arteriogenesis in mouse ischemic muscle [90] and enhanced tumor growth and neovascularization [91], suggesting that fine control of NF-κB activity is required to maintain normal vascularization.

3.2.3. Hypoxia-inducible factor (HIF)-1α

HIF-1 is an essential transcriptional factor in response to hypoxia and controls oxygen and nutrient delivery by promoting angiogenesis and vascular remodeling. It is a heterodimeric factor that is composed of an O_2-regulated HIF-1α and a constitutively expressed HIF-1β [92,93]. HIF-1α protein stability which is controlled by post-translational modification critically determines HIF-1 activity. HIF-1α hydroxylation at proline 402 and 564 in the oxygen-dependent degradation (ODD) domain by prolyl-hydroxylases (PHD) [94,95] is subjected to ubiquitination by E3 ubiquitin ligase, von Hippel Lindau protein (pVHL), and rapidly degraded by the proteasome. Under hypoxia, PHD activity and subsequent HIF-1α hydroxylation are attenuated, resulting in less degradation and accumulation of HIF-1α. In addition, NO-mediated *S*-nitrosylation at Cys^{533} (mouse, human Cys^{520} equivalent) in ODD domain of HIF-1α prevents degradation and enhances HIF-1 activity [96]. Furthermore, this cysteine may form the more stable GSH adduct. In fact, Glrx inhibition or increased GSH adducts *in vitro* stabilized HIF-1α protein by preventing interaction with pVHL, and mass spectrometry analysis recognized GSH adducts on Cys^{520} of HIF-1α. Glrx-/- mice *in vivo* increased overall GSH adducts, HIF-1α, and VEGF expression in ischemic muscles, and improved vascularization after hindlimb ischemia [49]. Increased GSH adducts in ischemic muscle may be a reflection of increased oxidants, but enhanced GSH adducts by endogenous Glrx deletion paradoxically promote recovery of ischemic limb *via* HIF-1α stabilization (Fig. 4). Previously reported ROS-dependent HIF-1α activation [97] may be explained by this mechanism of HIF-1α stabilization with GSH adducts.

3.2.4. Other targets of GSH adducts

Glrx overexpression is shown to protect EC barrier function *via* activation of small Rho GTPase Rac1 which is inactivated by GSH adducts under metabolic stress [98]. Decreased EC permeability by enhanced barrier function may also contribute to inhibition of angiogenesis by Glrx. There are other targets of GSH adducts which may not

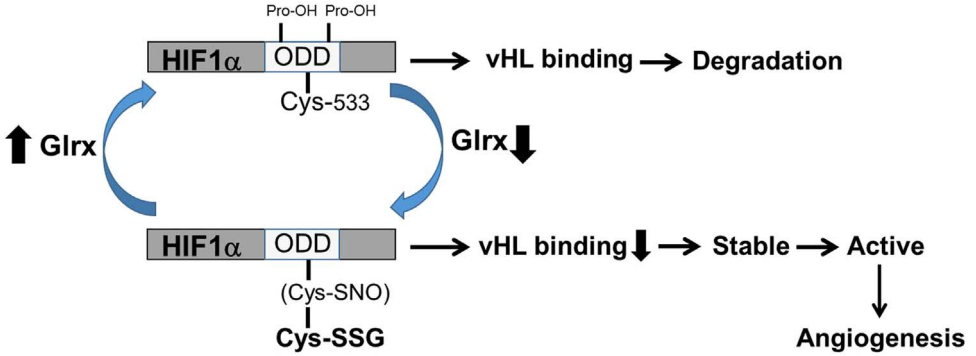

Fig. 4. Glutaredoxin controls HIF-1α stability. HIF-1α has a redox-sensitive cysteine (mouse Cys^{533}, human Cys^{520}) in oxygen-dependent degradation domain (ODD), which is modified by S-nitrosylation (-SNO) or GSH adducts (-SSG). The modified HIF-1α decreased binding to von Hippel Lindau protein (vHL), E3 ubiquitin ligase. Deletion or inhibition of Glrx can augment the modification with GSH adducts and stabilize HIF1α resulting in HIF-1 activation. Up-regulated Glrx removes GSH adducts and promotes HIF-1α degradation. Hydroxylation of proline (pro) residues in ODD usually causes vHL binding and HIF-1α degradation under normoxia. GSH adducts can be formed in hydroxylated HIF-1α in normoxia to prevent degradation [49].

promote vascularization. For instance, eNOS is known to be uncoupled by GSH adducts on critical cysteine residues [99]. Lower NO production by eNOS dysfunction is expected to inhibit angiogenesis, but increased superoxide anion or peroxynitrite may activate other angiogenic pathways. Glrx may target many proteins with GSH adducts, but *in vivo* Glrx contributes to the anti-angiogenic pathway as shown in Glrx-/- mice. Further mechanisms in which Glrx activates a certain signaling cascade in specific pathological model should be explored.

4. Summary and translational implication

In summary, studies using genetically modified mice indicate endogenous oxidants are essential to promote ischemic limb vascularization, which suggests caution should be taken for the therapeutic use of antioxidants since excess antioxidants may not only be ineffective but can be detrimental in the ischemic limb. There are several targets which oxidants activate angiogenic pathways by forming reversible thiol modification with GSH adducts such as HIF-1α□ Therefore, enhanced GSH adducts by inhibiting Glrx can promote ischemic limb vascularization.

Hind limb ischemia is used as a model for human peripheral artery disease (PAD). Clinical symptom of PAD is from acute reversible hypoperfusion which may cause intermittent claudication to critical limb ischemia which is chronic artery occlusion causing tissue necrosis [100]. Risk factors of PAD are age, smoking, and diabetes, and its pathogenesis is associated with atherosclerosis, endothelial dysfunction, and inflammation. Impaired angiogenesis and reduced microcirculation in the muscle of PAD patients potentiate limb ischemia [101]. Of interest critical limb ischemia patients have higher levels of plasma VEGF-A [102], indicating that VEGF downstream signaling may be affected in these patients. This may be incompatible with hindlimb ischemia of atherosclerotic animals which still respond exogenous VEGF [37,38]. Supervised exercise is recommended as effective therapy for PAD patients among other medical interventions [103,104]. This is supported by a mouse study which showed that exercise improved vascularization after femoral artery ligation dependent on inducible nitric oxide synthase (iNOS) in myeloid cells [105]. Oxidants derived from iNOS may promote vascularization and stimulate even tumor angiogenesis because high expression of iNOS relates to human cancer progression [106]. It should be noted, however, that PAD patients usually have a background of atherosclerosis or diabetes which may be associated with higher levels of oxidants and inflammation. These clinical conditions are not necessarily comparable with healthy young mice. Furthermore, stimulating angiogenesis may exacerbate atherosclerosis [107], suggesting complexity of treatment to PAD. High fat diet-fed mice [86] or hypercholesterolemic animals [37,38] show impaired vascularization after hindlimb ischemia. Future studies in such pathological models will be more translational to find therapeutic targets.

Disclosure

No disclosure.

Acknowledgments

We are very grateful for all support and mentorship which had been provided by Dr. Richard A. Cohen. This work was supported by National Institutes of Health (R01 HL133013, R03 AG051857), NIH CTSI award 1UL1TR001430, Banyu Foundation Research Grant, Diabetes UK (16/005453) and Marie-Curie International Incoming Fellowship (IVSCP 626633). We also thank Dr. Markus M. Bachschmid and Dr. Jingyan Han for their collaboration.

References

[1] P.M. Kris-Etherton, A.H. Lichtenstein, B.V. Howard, D. Steinberg, J.L. Witztum, Nutrition committee of the American Heart Association Council on nutrition, physical activity, and metabolism, antioxidant vitamin supplements and cardiovascular disease, Circulation 110 (2004) 637–641, http://dx.doi.org/10.1161/01.CIR.0000137822.39831.F1.

[2] Y. Watanabe, R.A. Cohen, R. Matsui, Redox regulation of ischemic angiogenesis-another aspect of reactive oxygen species, Circ. J. 80 (2016) 1278–1284, http://dx.doi.org/10.1253/circj.CJ-16-0317.

[3] Y.-W. Kim, T.V. Byzova, Oxidative stress in angiogenesis and vascular disease, Blood 123 (2014) 625–631, http://dx.doi.org/10.1182/blood-2013-09-512749.

[4] R. Colavitti, G. Pani, B. Bedogni, R. Anzevino, S. Borrello, J. Waltenberger, T. Galeotti, Reactive oxygen species as downstream mediators of angiogenic signaling by vascular endothelial growth factor receptor-2/KDR, J. Biol. Chem. 277 (2002) 3101–3108, http://dx.doi.org/10.1074/jbc.M107711200.

[5] T. Tojo, M. Ushio-Fukai, M. Yamaoka-Tojo, S. Ikeda, N. Patrushev, R.W. Alexander, Role of gp91phox (Nox2)-containing NAD(P)H oxidase in angiogenesis in response to hindlimb ischemia, Circulation 111 (2005) 2347–2355, http://dx.doi.org/10.1161/01.CIR.0000164261.62586.14.

[6] N. Urao, H. Inomata, M. Razvi, H.W. Kim, K. Wary, R. McKinney, T. Fukai, M. Ushio-Fukai, Role of nox2-based NADPH 0xidase in bone marrow and progenitor cell function involved in neovascularization induced by hindlimb ischemia, Circ. Res. 103 (2008) 212–220, http://dx.doi.org/10.1161/CIRCRESAHA.108.176230.

[7] S.M. Craige, K. Chen, Y. Pei, C. Li, X. Huang, C. Chen, R. Shibata, K. Sato, K. Walsh, J.F. Keaney, NADPH oxidase 4 promotes endothelial angiogenesis through endothelial nitric oxide synthase activation, Circulation 124 (2011) 731–740, http://dx.doi.org/10.1161/CIRCULATIONAHA.111.030775.

[8] L. Chen, X. Hou, J. Xiao, J. Kuroda, T. Ago, J. Sadoshima, R.A. Cohen, X.Y. Tong, Both hydrogen peroxide and transforming growth factor beta 1 contribute to endothelial Nox4 mediated angiogenesis in endothelial Nox4 transgenic mouse lines, Biochim. Biophys. Acta – Mol. Basis Dis. 1842 (2014) 2489–2499, http://dx.doi.org/10.1016/j.bbadis.2014.10.007.

[9] R.S. Sohal, R.J. Mockett, W.C. Orr, Mechanisms of aging: an appraisal of the oxidative stress hypothesis, Free Radic. Biol. Med. 33 (2002) 575–586.

[10] A.B. Hwang, E.-A. Ryu, M. Artan, H.-W. Chang, M.H. Kabir, H.-J. Nam, D. Lee, J.-S. Yang, S. Kim, W.B. Mair, C. Lee, S.S. Lee, S.-J. Lee, Feedback regulation via

AMPK and HIF-1 mediates ROS-dependent longevity in Caenorhabditis elegans, Proc. Natl. Acad. Sci. USA 111 (2014) E4458–E4467, http://dx.doi.org/10.1073/pnas.1411199111.
[11] M. Ushio-Fukai, Redox signaling in angiogenesis: role of NADPH oxidase, Cardiovasc. Res. 71 (2006) 226–235, http://dx.doi.org/10.1016/j.cardiores.2006.04.015.
[12] R.S. Frey, M. Ushio-Fukai, A. Malik, NADPH oxidase-dependent signaling in endothelial cells: role in physiology and pathophysiology, Antioxid. Redox Signal. 11 (2009) 791–810, http://dx.doi.org/10.1089/ars.2008.2220.
[13] J.J. Mieyal, M.M. Gallogly, S. Qanungo, E.A. Sabens, M.D. Shelton, Molecular mechanisms and clinical implications of reversible protein S-glutathionylation, Antioxid. Redox Signal. 10 (2008) 1941–1988, http://dx.doi.org/10.1089/ars.2008.2089.
[14] D. Pimentel, D.J. Haeussler, R. Matsui, J.R. Burgoyne, R.A. Cohen, M.M. Bachschmid, Regulation of cell physiology and pathology by protein S-glutathionylation: lessons learned from the cardiovascular system, Antioxid. Redox Signal. 16 (2012) 524–542, http://dx.doi.org/10.1089/ars.2011.4336.
[15] U. Florczyk, A. Jazwa, M. Maleszewska, M. Mendel, K. Szade, M. Kozakowska, A. Grochot-Przeczek, M. Viscardi, S. Czauderna, K. Bukowska-Strakova, J. Kotlinowski, A. Jozkowicz, A. Loboda, J. Dulak, Nrf2 regulates angiogenesis: effect on endothelial cells, bone marrow-derived proangiogenic cells and hind limb ischemia, Antioxid. Redox Signal. 20 (2014) 1693–1708, http://dx.doi.org/10.1089/ars.2013.5219.
[16] M. Heil, I. Eitenmüller, T. Schmitz-Rixen, W. Schaper, Arteriogenesis versus angiogenesis: similarities and differences, J. Cell. Mol. Med. 10 (2006) 45–55, http://dx.doi.org/10.1111/j.1582-4934.2006.tb00290.x.
[17] E. Wahlberg, Angiogenesis and arteriogenesis in limb ischemia, J. Vasc. Surg. 38 (2003) 198–203, http://dx.doi.org/10.1016/S0741-5214(03)00151-4.
[18] M. Simons, K. Alitalo, B.H. Annex, H.G. Augustin, C. Beam, B.C. Berk, T. Byzova, P. Carmeliet, W. Chilian, J.P. Cooke, G.E. Davis, A. Eichmann, M.L. Iruela-Arispe, E. Keshet, A.J. Sinusas, C. Ruhrberg, Y.J. Woo, S. Dimmeler, American Heart Association Council on basic cardiovascular sciences and council on cardiovascular surgery and anesthesia, state-of-the-art methods for evaluation of angiogenesis and tissue vascularization: a scientific statement from the American Heart Association, Circ. Res. 116 (2015) e99–e132, http://dx.doi.org/10.1161/RES.0000000000000054.
[19] A. Limbourg, T. Korff, L.C. Napp, W. Schaper, H. Drexler, F.P. Limbourg, Evaluation of postnatal arteriogenesis and angiogenesis in a mouse model of hind-limb ischemia, Nat. Protoc. 4 (2009) 1737–1746, http://dx.doi.org/10.1038/nprot.2009.185.
[20] T. Murohara, T. Asahara, M. Silver, C. Bauters, H. Masuda, C. Kalka, M. Kearney, D. Chen, D. Chen, J.F. Symes, M.C. Fishman, P.L. Huang, J.M. Isner, Nitric oxide synthase modulates angiogenesis in response to tissue ischemia, J. Clin. Invest. 101 (1998) 2567–2578, http://dx.doi.org/10.1172/JCI1560.
[21] R. Ray, C.E. Murdoch, M. Wang, C.X. Santos, M. Zhang, S. Alom-Ruiz, N. Anilkumar, A. Ouattara, A.C. Cave, S.J. Walker, D.J. Grieve, R.L. Charles, P. Eaton, A.C. Brewer, A.M. Shah, Endothelial Nox4 NADPH oxidase enhances vasodilatation and reduces blood pressure in vivo, Arterioscler. Thromb. Vasc. Biol. 31 (2011) 1368–1376, http://dx.doi.org/10.1161/ATVBAHA.110.219238.
[22] K. Schröder, M. Zhang, S. Benkhoff, A. Mieth, R. Pliquett, J. Kosowski, C. Kruse, P. Luedike, U.R. Michaelis, N. Weissmann, S. Dimmeler, A.M. Shah, R.P. Brandes, Nox4 is a protective reactive oxygen species generating vascular NADPH oxidase, Circ. Res. 110 (2012) 1217–1225, http://dx.doi.org/10.1161/CIRCRESAHA.112.267054.
[23] N. Urao, V. Sudhahar, S.-J. Kim, G.-F. Chen, R.D. McKinney, G. Kojda, T. Fukai, M. Ushio-Fukai, Critical role of endothelial hydrogen peroxide in post-ischemic neovascularization, PLoS One 8 (2013) e57618, http://dx.doi.org/10.1371/journal.pone.0057618.
[24] R. Hodara, D. Weiss, G. Joseph, J.C. Velasquez-Castano, N. Landázuri, J.W. Han, Y.S. Yoon, W.R. Taylor, Overexpression of catalase in myeloid cells causes impaired postischemic neovascularization, Arterioscler. Thromb. Vasc. Biol. 31 (2011) 2203–2209, http://dx.doi.org/10.1161/ATVBAHA.111.233247.
[25] H.W. Kim, A. Lin, R.E. Guldberg, M. Ushio-Fukai, T. Fukai, Essential role of extracellular SOD in reparative neovascularization induced by hindlimb ischemia, Circ. Res. 101 (2007) 409–419, http://dx.doi.org/10.1161/CIRCRESAHA.107.153791.
[26] J. Oshikawa, N. Urao, H.W. Kim, N. Kaplan, M. Razvi, R. McKinney, L.B. Poole, T. Fukai, M. Ushio-Fukai, Extracellular SOD-derived H2O2 promotes VEGF signaling in caveolae/lipid rafts and post-ischemic angiogenesis in mice, PLoS One 5 (2010), http://dx.doi.org/10.1371/journal.pone.0010189.
[27] S. Ichihara, Y. Yamada, F. Liu, T. Murohara, K. Itoh, M. Yamamoto, G. Ichihara, Ablation of the transcription factor Nrf2 promotes ischemia-induced neovascularization by enhancing the inflammatory response, Arterioscler. Thromb. Vasc. Biol. 30 (2010) 1553–1561, http://dx.doi.org/10.1161/ATVBAHA.110.204123.
[28] T.P. Dalton, M.Z. Dieter, Y. Yang, H.G. Shertzer, D.W. Nebert, Knockout of the mouse glutamate cysteine ligase catalytic subunit (Gclc) gene: embryonic lethal when homozygous, and proposed model for moderate glutathione deficiency when heterozygous, Biochem. Biophys. Res. Commun. 279 (2000) 324–329, http://dx.doi.org/10.1006/bbrc.2000.3930.
[29] T. Kobayashi, Y. Watanabe, Y. Saito, D. Fujioka, T. Nakamura, J.E. Obata, Y. Kitta, T. Yano, K. Kawabata, K. Watanabe, H. Mishina, S. Ito, K. Kugiyama, Mice lacking the glutamate-cysteine ligase modifier subunit are susceptible to myocardial ischaemia-reperfusion injury, Cardiovasc. Res. 85 (2010) 785–795, http://dx.doi.org/10.1093/cvr/cvp342.
[30] Y. Watanabe, K. Watanabe, T. Kobayashi, Y. Saito, D. Fujioka, T. Nakamura, J.E. Obata, K. Kawabata, H. Mishina, K. Kugiyama, Chronic depletion of glutathione exacerbates ventricular remodelling and dysfunction in the pressure-overloaded heart, Cardiovasc. Res. 97 (2013) 282–292, http://dx.doi.org/10.1093/cvr/cvs333.
[31] S.C. Bir, X. Shen, T.J. Kavanagh, C.G. Kevil, C.B. Pattillo, Control of angiogenesis dictated by picomolar superoxide levels, Free Radic. Biol. Med. 63 (2013) 135–142, http://dx.doi.org/10.1016/j.freeradbiomed.2013.05.015.
[32] P. Haddad, S. Dussault, J. Groleau, J. Turgeon, S.E. Michaud, C. Ménard, G. Perez, F. Maingrette, A. Rivard, Nox2-containing NADPH oxidase deficiency confers protection from hindlimb ischemia in conditions of increased oxidative stress, Arterioscler. Thromb. Vasc. Biol. 29 (2009) 1522–1528, http://dx.doi.org/10.1161/ATVBAHA.109.191437.
[33] P. Haddad, S. Dussault, J. Groleau, J. Turgeon, F. Maingrette, A. Rivard, Nox2-derived reactive oxygen species contribute to hypercholesterolemia-induced inhibition of neovascularization: effects on endothelial progenitor cells and mature endothelial cells, Atherosclerosis 217 (2011) 340–349, http://dx.doi.org/10.1016/j.atherosclerosis.2011.03.038.
[34] T.G. Ebrahimian, C. Heymes, D. You, O. Blanc-Brude, B. Mees, L. Waeckel, M. Duriez, J. Vilar, R.P. Brandes, B.I. Levy, A.M. Shah, J.-S. Silvestre, NADPH oxidase-derived overproduction of reactive oxygen species impairs postischemic neovascularization in mice with type 1 diabetes, Am. J. Pathol. 169 (2006) 719–728, http://dx.doi.org/10.2353/ajpath.2006.060042.
[35] T.E. Ryan, C.A. Schmidt, T.D. Green, E.E. Spangenburg, P.D. Neufer, J.M. McClung, Targeted expression of catalase to mitochondria protects against ischemic myopathy in high-fat diet-fed mice, Diabetes 65 (2016) 2553–2568, http://dx.doi.org/10.2337/db16-0387.
[36] S. Dussault, F. Maingrette, C. Ménard, S.É. Michaud, P. Haddad, J. Groleau, J. Turgeon, G. Perez, A. Rivard, Sildenafil increases endothelial progenitor cell function and improves ischemia-induced neovascularization in hypercholesterolemic apolipoprotein E-deficient mice, Hypertension 54 (2009) 1043–1049, http://dx.doi.org/10.1161/HYPERTENSIONAHA.109.139451.
[37] T. Couffinhal, M. Silver, M. Kearney, A. Sullivan, B. Witzenbichler, M. Magner, B. Annex, K. Peters, J.M. Isner, Impaired collateral vessel development associated with reduced expression of vascular endothelial growth factor in ApoE-/- mice, Circulation 99 (1999) 3188–3198, http://dx.doi.org/10.1161/01.CIR.99.24.3188.
[38] E. Van Belle, A. Rivard, D. Chen, M. Silver, S. Bunting, N. Ferrara, J.F. Symes, C. Bauters, J.M. Isner, Hypercholesterolemia attenuates angiogenesis but does not preclude augmentation by angiogenic cytokines, Circulation 96 (1997) 2667–2674.
[39] A. Holmgren, Hydrogen donor system for Escherichia coli ribonucleoside-diphosphate reductase dependent upon glutathione, Proc. Natl. Acad. Sci. USA 73 (1976) 2275–2279, http://dx.doi.org/10.1073/pnas.73.7.2275.
[40] A.P. Fernandes, A. Holmgren, Glutaredoxins: glutathione-dependent redox enzymes with functions far beyond a simple thioredoxin backup system, Antioxid. Redox Signal. 6 (2004) 63–74.
[41] S. Dai, Y. He, H. Zhang, L. Yu, T. Wan, Z. Xu, D. Jones, H. Chen, W. Min, Endothelial-specific expression of mitochondrial thioredoxin promotes ischemia-mediated arteriogenesis and angiogenesis, Arterioscler. Thromb. Vasc. Biol. 29 (2009) 495–502, http://dx.doi.org/10.1161/ATVBAHA.108.180349.
[42] L.L. Dunn, A.M. Buckle, J.P. Cooke, M.K.C. Ng, The emerging role of the thioredoxin system in angiogenesis, Arterioscler. Thromb. Vasc. Biol. 30 (2010) 2089–2098, http://dx.doi.org/10.1161/ATVBAHA.110.209643.
[43] R.S. Adluri, M. Thirunavukkarasu, L. Zhan, Y. Akita, S.M. Samuel, H. Otani, Y.-S. Ho, G. Maulik, N. Maulik, Thioredoxin 1 enhances neovascularization and reduces ventricular remodeling during chronic myocardial infarction: a study using thioredoxin 1 transgenic mice, J. Mol. Cell. Cardiol. 50 (2011) 239–247, http://dx.doi.org/10.1016/j.yjmcc.2010.11.002.
[44] V. Perez, V.D. Annunzio, L.B. Valdez, T. Zaobornyj, S. Bombicino, T. Mazo, N.L. Carbajosa, M.M. Gironacci, A. Boveris, J. Sadoshima, R.J. Gelpi, Thioredoxin-1 attenuates ventricular and mitochondrial post-ischemic dysfunction in the stunned myocardium of transgenic mice, Antioxid. Redox Signal. 25 (2016) 1–33, http://dx.doi.org/10.1089/ars.2015.6459.
[45] L.L. Dunn, P.J.L. Simpson, H.C. Prosser, L. Lecce, G.S.C. Yuen, A. Buckle, D.P. Sieveking, L.Z. Vanags, P.R. Lim, R.W.Y. Chow, Y.T. Lam, Z. Clayton, S. Bao, M.J. Davies, N. Stadler, D.S. Celermajer, R. Stocker, C.A. Bursill, J.P. Cooke, M.K.C. Ng, A critical role for thioredoxin-interacting protein in diabetes-related impairment of angiogenesis, Diabetes 63 (2014) 675–687, http://dx.doi.org/10.2337/db13-0417.
[46] M. Lundberg, C. Johansson, J. Chandra, M. Enoksson, G. Jacobsson, J. Ljung, M. Johansson, a. Holmgren, Cloning and expression of a novel human glutaredoxin (Grx2) with mitochondrial and nuclear isoforms, J. Biol. Chem. 276 (2001) 26269–26275, http://dx.doi.org/10.1074/jbc.M011605200.
[47] A.M. Evangelista, M.D. Thompson, R.M. Weisbrod, D.R. Pimental, X. Tong, V.M. Bolotina, R.A. Cohen, Redox regulation of SERCA2 is required for vascular endothelial growth factor-induced signaling and endothelial cell migration, Antioxid. Redox Signal. 17 (2012) 1099–1108, http://dx.doi.org/10.1089/ars.2011.4022.
[48] C.E. Murdoch, M. Shuler, D.J.F. Haeussler, R. Kikuchi, P. Bearelly, J. Han, Y. Watanabe, J.J. Fuster, K. Walsh, Y.-S. Ho, M.M. Bachschmid, R.A. Cohen, R. Matsui, Glutaredoxin-1 up-regulation induces soluble vascular endothelial growth factor receptor 1, attenuating post-ischemia limb revascularization, J. Biol. Chem. 289 (2014) 8633–8644, http://dx.doi.org/10.1074/jbc.M113.517219.
[49] Y. Watanabe, C.E. Murdoch, S. Sano, Y. Ido, M.M. Bachschmid, R.A. Cohen, R. Matsui, Glutathione adducts induced by ischemia and deletion of glutaredoxin-1 stabilize HIF-1α and improve limb revascularization, Proc. Natl. Acad. Sci. USA (2016) 1524198113, http://dx.doi.org/10.1073/pnas.1524198113.
[50] Y.-S. Ho, Y. Xiong, D.S. Ho, J. Gao, B.H.L. Chua, H. Pai, J.J. Mieyal, Targeted

disruption of the glutaredoxin 1 gene does not sensitize adult mice to tissue injury induced by ischemia/reperfusion and hyperoxia, Free Radic. Biol. Med. 43 (2007) 1299–1312, http://dx.doi.org/10.1016/j.freeradbiomed.2007.07.025.
[51] M.M. Bachschmid, S. Xu, K.A. Maitland-Toolan, Y.-S. Ho, R.A. Cohen, R. Matsui, Attenuated cardiovascular hypertrophy and oxidant generation in response to angiotensin II infusion in glutaredoxin-1 knockout mice, Free Radic. Biol. Med. 49 (2010) 1221–1229, http://dx.doi.org/10.1016/j.freeradbiomed.2010.07.005.
[52] R.A. Cohen, C.E. Murdoch, Y. Watanabe, V.M. Bolotina, A.M. Evangelista, D.J. Haeussler, M.D. Smith, Y. Mei, X. Tong, J. Han, J.B. Behring, M.M. Bachschmid, R. Matsui, Endothelial cell redox regulation of ischemic angiogenesis, J. Cardiovasc. Pharmacol. 67 (2016) 458–464, http://dx.doi.org/10.1097/FJC.0000000000000381.
[53] Y.H. Looi, D.J. Grieve, A. Siva, S.J. Walker, N. Anilkumar, A.C. Cave, M. Marber, M.J. Monaghan, A.M. Shah, Involvement of Nox2 NADPH oxidase in adverse cardiac remodeling after myocardial infarction, Hypertension 51 (2008) 319–325, http://dx.doi.org/10.1161/HYPERTENSIONAHA.107.101980.
[54] C. Doerries, K. Grote, D. Hilfiker-Kleiner, M. Luchtefeld, A. Schaefer, S.M. Holland, S. Sorrentino, C. Manes, B. Schieffer, H. Drexler, U. Landmesser, Critical role of the NAD(P)H oxidase subunit p47phox for left ventricular remodeling/dysfunction and survival after myocardial infarction, Circ. Res. 100 (2007) 894–903, http://dx.doi.org/10.1161/01.RES.0000261657.76299.ff.
[55] A. Sirker, C.E. Murdoch, A. Protti, G.J. Sawyer, C.X.C. Santos, D. Martin, X. Zhang, A.C. Brewer, M. Zhang, A.M. Shah, Cell-specific effects of Nox2 on the acute and chronic response to myocardial infarction, J. Mol. Cell. Cardiol. 98 (2016) 11–17, http://dx.doi.org/10.1016/j.yjmcc.2016.07.003.
[56] C.E. Murdoch, S. Chaubey, L. Zeng, B. Yu, A. Ivetic, S.J. Walker, D. Vanhoutte, S. Heymans, D.J. Grieve, A.C. Cave, A.C. Brewer, M. Zhang, A.M. Shah, Endothelial NADPH oxidase-2 promotes interstitial cardiac fibrosis and diastolic dysfunction through proinflammatory effects and endothelial- mesenchymal transition, J. Am. Coll. Cardiol. 63 (2014) 2734–2741, http://dx.doi.org/10.1016/j.jacc.2014.02.572.
[57] E. Bernadette Cabigas, I. Somasuntharam, M.E. Brown, P.L. Che, K.D. Pendergrass, B. Chiang, W. Robert Taylor, M.E. Davis, Over-expression of catalase in myeloid cells confers acute protection following myocardial infarction, Int. J. Mol. Sci. 15 (2014) 9036–9050, http://dx.doi.org/10.3390/ijms15059036.
[58] R.S. Adluri, M. Thirunavukkarasu, L. Zhan, N.R. Dunna, Y. Akita, V. Selvaraju, H. Otani, J.A. Sanchez, Y.-S. Ho, N. Maulik, Glutaredoxin-1 overexpression enhances neovascularization and diminishes ventricular remodeling in chronic myocardial infarction, PLoS One 7 (2012) e34790, http://dx.doi.org/10.1371/journal.pone.0034790.
[59] P. Klatt, S. Lamas, Regulation of protein function by S-glutathiolation in response to oxidative and nitrosative stress, Eur. J. Biochem. 267 (2000) 4928–4944.
[60] B. Huang, C. Chen, An ascorbate-dependent artifact that interferes with the interpretation of the biotin switch assay, Free Radic. Biol. Med. 41 (2006) 562–567.
[61] A. Martínez-Ruiz, S. Lamas, Signalling by NO-induced protein S-nitrosylation and S-glutathionylation: convergences and divergences, Cardiovasc. Res. 75 (2007) 220–228, http://dx.doi.org/10.1016/j.cardiores.2007.03.016.
[62] D.M. Townsend, Y. Manevich, H. Lin, X. Ying, R.R. Bowers, S. Hutchens, K.D. Tew, Nitrosative stress-induced S-glutathionylation of protein disulfide isomerase leads to activation of the unfolded protein response, Cancer Res. 69 (2009) 7626–7634, http://dx.doi.org/10.1158/0008-5472.CAN-09-0493.
[63] J.D. Uys, Y. Xiong, D.M. Townsend, Nitrosative stress-induced S-glutathionylation of protein disulfide isomerase, Methods Enzymol. 490 (2011) 321–332, http://dx.doi.org/10.1016/B978-0-12-385114-7.00018-0.
[64] D.W. Starke, P.B. Chock, J.J. Mieyal, Glutathione-thiyl radical scavenging and transferase properties of human glutaredoxin (Thioltransferase): potential role in redox signal transduction, J. Biol. Chem. 278 (2003) 14607–14613, http://dx.doi.org/10.1074/jbc.M210434200.
[65] S. Qanungo, D.W. Starke, H.V. Pai, J.J. Mieyal, A.-L. Nieminen, Glutathione supplementation potentiates hypoxic apoptosis by S-glutathionylation of p65-NFkappaB, J. Biol. Chem. 282 (2007) 18427–18436, http://dx.doi.org/10.1074/jbc.M610934200.
[66] D.M. Townsend, Y. Manevich, L. He, S. Hutchens, C.J. Pazoles, K.D. Tew, Novel role for glutathione S-transferase pi. Regulator of protein S-Glutathionylation following oxidative and nitrosative stress, J. Biol. Chem. 284 (2009) 436–445, http://dx.doi.org/10.1074/jbc.M805586200.
[67] D.H. McMillan, J.L.J. van der Velden, K.G. Lahue, X. Qian, R.W. Schneider, M.S. Iberg, J.D. Nolin, S. Abdalla, D.T. Casey, K.D. Tew, D.M. Townsend, C.J. Henderson, C.R. Wolf, K.J. Butnor, D.J. Taatjes, R.C. Budd, C.G. Irvin, A. van der Vliet, S. Flemer, V. Anathy, Y.M.W. Janssen-Heininger, Attenuation of lung fibrosis in mice with a clinically relevant inhibitor of glutathione-S-transferase π, JCI Insight 1 (2016) 1–16, http://dx.doi.org/10.1172/jci.insight.85717.
[68] R.A. Cohen, T. Adachi, Nitric-oxide-induced vasodilatation: regulation by physiologic S-glutathiolation and pathologic oxidation of the sarcoplasmic endoplasmic reticulum calcium ATPase, Trends Cardiovasc. Med. 16 (2006) 109–114, http://dx.doi.org/10.1016/j.tcm.2006.02.001.
[69] J. Wang, E.S. Boja, W. Tan, E. Tekle, H.M. Fales, S. English, J.J. Mieyal, P.B. Chock, Reversible glutathionylation regulates actin polymerization in A431 cells, J. Biol. Chem. 276 (2001) 47763–47766, http://dx.doi.org/10.1074/jbc.C100415200.
[70] A.N. Carr, M.G. Davis, E. Eby-Wilkens, B.W. Howard, B.A. Towne, T.E. Dufresne, K.G. Peters, Tyrosine phosphatase inhibition augments collateral blood flow in a rat model of peripheral vascular disease, Am. J. Physiol. Heart Circ. Physiol. 287 (2004) 268–276, http://dx.doi.org/10.1152/ajpheart.00007.2004.
[71] Y. Nakamura, N. Patrushev, H. Inomata, D. Mehta, N. Urao, H.W. Kim, M. Razvi, V. Kini, K. Mahadev, B.J. Goldstein, R. McKinney, T. Fukai, M. Ushio-Fukai, Role of protein tyrosine phosphatase 1B in vascular endothelial growth factor signaling and cell-cell adhesions in endothelial cells, Circ. Res. 102 (2008) 1182–1191, http://dx.doi.org/10.1161/CIRCRESAHA.107.167080.
[72] W.C. Barrett, J.P. DeGnore, Y.F. Keng, Z.Y. Zhang, M.B. Yim, P.B. Chock, Roles of superoxide radical anion in signal transduction mediated by reversible regulation of protein-tyrosine phosphatase 1B, J. Biol. Chem. 274 (1999) 34543–34546.
[73] L. Huang, S. Sankar, C. Lin, C.D. Kontos, A.D. Schroff, E.H. Cha, S.M. Feng, S.F. Li, Z. Yu, R.L. Van Etten, M.A. Blanar, K.G. Peters, HCPTPA, a protein tyrosine phosphatase that regulates vascular endothelial growth factor receptor-mediated signal transduction and biological activity, J. Biol. Chem. 274 (1999) 38183–38188, http://dx.doi.org/10.1074/JBC.274.53.38183.
[74] M.A. Abdelsaid, A.B. El-Remessy, S-glutathionylation of LMW-PTP regulates VEGF-mediated FAK activation and endothelial cell migration, J. Cell Sci. 125 (2012) 4751–4760, http://dx.doi.org/10.1242/jcs.103481.
[75] M. Besnier, A. Galaup, L. Nicol, J.P. Henry, D. Coquerel, A. Gueret, P. Mulder, E. Brakenhielm, C. Thuillez, S. Germain, V. Richard, A. Ouvrard-Pascaud, Enhanced angiogenesis and increased cardiac perfusion after myocardial infarction in protein tyrosine phosphatase 1B-deficient mice, FASEB J. 28 (2014) 3351–3361, http://dx.doi.org/10.1096/fj.13-245753.
[76] A.A. Lanahan, D. Lech, A. Dubrac, J. Zhang, Z.W. Zhuang, A. Eichmann, M. Simons, PTP1b is a physiologic regulator of vascular endothelial growth factor signaling in endothelial cells, Circulation 130 (2014) 902–909, http://dx.doi.org/10.1161/CIRCULATIONAHA.114.009683.
[77] T. Adachi, R.M. Weisbrod, D.R. Pimentel, J. Ying, V.S. Sharov, C. Schöneich, R.A. Cohen, S-Glutathiolation by peroxynitrite activates SERCA during arterial relaxation by nitric oxide, Nat. Med. 10 (2004) 1200–1207, http://dx.doi.org/10.1038/nm1119.
[78] M.D. Thompson, Y. Mei, R.M. Weisbrod, M. Silver, P.C. Shukla, V.M. Bolotina, R.A. Cohen, X. Tong, Glutathione adducts on sarcoplasmic/endoplasmic reticulum Ca2+ ATPase Cys-674 regulate endothelial cell calcium stores and angiogenic function as well as promote ischemic blood flow recovery, J. Biol. Chem. 289 (2014) 19907–19916, http://dx.doi.org/10.1074/jbc.M114.554451.
[79] Y. Mei, M.D. Thompson, Y. Shiraishi, R.A. Cohen, X. Tong, Sarcoplasmic/endoplasmic reticulum Ca2+ ATPase C674 promotes ischemia- and hypoxia-induced angiogenesis via coordinated endothelial cell and macrophage function, J. Mol. Cell. Cardiol. 76 (2014) 275–282, http://dx.doi.org/10.1016/j.yjmcc.2014.09.018.
[80] N.L. Reynaert, A. van der Vliet, A.S. Guala, T. McGovern, M. Hristova, C. Pantano, N.H. Heintz, J. Heim, Y.-S. Ho, D.E. Matthews, Dynamic redox control of NF-κB through glutaredoxin-regulated S-glutathionylation of inhibitory κB kinase β, Proc. Natl. Acad. Sci. USA 103 (2006) 13086–13091.
[81] E. Pineda-Molina, P. Klatt, J. Vázquez, A. Marina, M. García de Lacoba, D. Pérez-Sala, S. Lamas, Glutathionylation of the p50 subunit of NF-kappaB: a mechanism for redox-induced inhibition of DNA binding, Biochemistry 40 (2001) 14134–14142.
[82] M.D. Shelton, T.S. Kern, J.J. Mieyal, Glutaredoxin regulates nuclear factor kappa-B and intercellular adhesion molecule in Müller cells: model of diabetic retinopathy, J. Biol. Chem. 282 (2007) 12467–12474, http://dx.doi.org/10.1074/jbc.M610863200.
[83] O.G. Miller, J.B. Behring, S.L. Siedlak, S. Jiang, R. Matsui, M.M. Bachschmid, X. Zhu, J.J. Mieyal, Upregulation of glutaredoxin-1 activates microglia and promotes neurodegeneration: implications for Parkinson's disease, Antioxid. Redox Signal. 25 (2016) 967–982, http://dx.doi.org/10.1089/ars.2015.6598.
[84] R.L. Kendall, K.A. Thomas, Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor, Proc. Natl. Acad. Sci. USA 90 (1993) 10705–10709.
[85] J.A. Stefater, I. Lewkowich, S. Rao, G. Mariggi, A.C. Carpenter, A.R. Burr, J. Fan, R. Ajima, J.D. Molkentin, B.O. Williams, M. Wills-Karp, J.W. Pollard, T. Yamaguchi, N. Ferrara, H. Gerhardt, R.A. Lang, Regulation of angiogenesis by a non-canonical Wnt-Flt1 pathway in myeloid cells, Nature 474 (2011) 511–515, http://dx.doi.org/10.1038/nature10085.
[86] S. Hazarika, A.O. Dokun, Y. Li, A.S. Popel, C.D. Kontos, B.H. Annex, Impaired angiogenesis after hindlimb ischemia in type 2 diabetes mellitus: differential regulation of vascular endothelial growth factor receptor 1 and soluble vascular endothelial growth factor receptor 1, Circ. Res. 101 (2007) 948–956, http://dx.doi.org/10.1161/CIRCRESAHA.107.160630.
[87] S.W. Aesif, I. Kuipers, J. van der Velden, J.E. Tully, A.S. Guala, V. Anathy, J.I. Sheely, N.L. Reynaert, E.F.M. Wouters, A. van der Vliet, Y.M.W. Janssen-Heininger, Activation of the glutaredoxin-1 gene by nuclear factor κB enhances signaling, Free Radic. Biol. Med. 51 (2011) 1249–1257, http://dx.doi.org/10.1016/j.freeradbiomed.2011.06.025.
[88] C.E. Tabit, S.M. Shenouda, M. Holbrook, J.L. Fetterman, S. Kiani, A.A. Frame, M.A. Kluge, A. Held, M.M. Dohadwala, N. Gokce, M.G. Farb, J. Rosenzweig, N. Ruderman, J.A. Vita, N.M. Hamburg, Protein kinase C- contributes to impaired endothelial insulin signaling in humans with diabetes mellitus, Circulation 127 (2013) 86–95, http://dx.doi.org/10.1161/CIRCULATIONAHA.112.127514.
[89] H. Carlsen, F. Haugen, S. Zadelaar, R. Kleemann, T. Kooistra, C.A. Drevon, R. Blomhoff, Diet-induced obesity increases NF-κB signaling in reporter mice, Genes Nutr. 4 (2009) 215–222, http://dx.doi.org/10.1007/s12263-009-0133-6.
[90] D. Tirziu, I.M. Jaba, P. Yu, B. Larrivée, B.G. Coon, B. Cristofaro, Z.W. Zhuang, A.A. Lanahan, M.A. Schwartz, A. Eichmann, M. Simons, Endothelial nuclear factor-κB-dependent regulation of arteriogenesis and branching, Circulation 126 (2012) 2589–2600, http://dx.doi.org/10.1161/CIRCULATIONAHA.112.119321.
[91] T. Kisseleva, L. Song, M. Vorontchikhina, N. Feirt, J. Kitajewski, C. Schindler, NF-κB regulation of endothelial cell function during LPS-induced toxemia and cancer,

J. Clin. Investig. 116 (2006) 2955–2963, http://dx.doi.org/10.1172/JCI27392.
[92] G.L. Wang, G.L. Semenza, Purification and characterization of hypoxia-inducible factor 1, J. Biol. Chem. 270 (1995) 1230–1237 (doi:7836384).
[93] G.L. Semenza, Hypoxia-inducible factor 1 and cardiovascular disease, Annu. Rev. Physiol. 76 (2014) 39–56, http://dx.doi.org/10.1146/annurev-physiol-021113-170322.
[94] M. Ohh, C.W. Park, M. Ivan, M.A. Hoffman, T.Y. Kim, L.E. Huang, N. Pavletich, V. Chau, W.G. Kaelin, Ubiquitination of hypoxia-inducible factor requires direct binding to the beta-domain of the von Hippel-Lindau protein, Nat. Cell Biol. 2 (2000) 423–427, http://dx.doi.org/10.1038/35017054.
[95] P. Jaakkola, D.R. Mole, Y.M. Tian, M.I. Wilson, J. Gielbert, S.J. Gaskell, A.V. Kriegsheim, H.F. Hebestreit, M. Mukherji, C.J. Schofield, P.H. Maxwell, C.W. Pugh, P.J. Ratcliffe, Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation, Science (80-.) 292 (2001) 468–472, http://dx.doi.org/10.1126/science.1059796.
[96] F. Li, P. Sonveaux, Z.N. Rabbani, S. Liu, B. Yan, Q. Huang, Z. Vujaskovic, M.W. Dewhirst, C.Y. Li, Regulation of HIF-1α stability through S-nitrosylation, Mol. Cell 26 (2007) 63–74, http://dx.doi.org/10.1016/j.molcel.2007.02.024.
[97] N.S. Chandel, E. Maltepe, E. Goldwasser, C.E. Mathieu, M.C. Simon, P.T. Schumacker, Mitochondrial reactive oxygen species trigger hypoxia-induced transcription, Proc. Natl. Acad. Sci. USA 95 (1998) 11715–11720, http://dx.doi.org/10.1073/pnas.95.20.11715.
[98] J. Han, R.M. Weisbrod, D. Shao, Y. Watanabe, X. Yin, M.M. Bachschmid, F. Seta, Y.M.W. Janssen-Heininger, R. Matsui, M. Zang, N.M. Hamburg, R.A. Cohen, The redox mechanism for vascular barrier dysfunction associated with metabolic disorders: glutathionylation of Rac1 in endothelial cells, Redox Biol. 9 (2016) 306–319, http://dx.doi.org/10.1016/j.redox.2016.09.003.
[99] C.-A. Chen, T.-Y. Wang, S. Varadharaj, L.A. Reyes, C. Hemann, M.A.H. Talukder, Y.-R. Chen, L.J. Druhan, J.L. Zweier, S-glutathionylation uncouples eNOS and regulates its cellular and vascular function, Nature 468 (2010) 1115–1118, http://dx.doi.org/10.1038/nature09599.
[100] M.D. Gerhard-Herman, H.L. Gornik, C. Barrett, N.R. Barshes, M.A. Corriere, D.E. Drachman, L.A. Fleisher, F.G.R. Fowkes, N.M. Hamburg, S. Kinlay, R. Lookstein, S. Misra, L. Mureebe, J.W. Olin, R.A.G. Patel, J.G. Regensteiner, A. Schanzer, M.H. Shishehbor, K.J. Stewart, D. Treat-Jacobson, M.E. Walsh, 2016 AHA/ACC guideline on the management of patients with lower extremity peripheral artery disease: executive summary: a report of the American College of Cardiology/American Heart Association task force on clinical practice guidelines, Circulation 135 (2017) e686–e725, http://dx.doi.org/10.1161/CIR.0000000000000470.
[101] N.M. Hamburg, M.A. Creager, Pathophysiology of intermittent claudication in peripheral artery disease, Circ. J. 81 (2017) 281–289, http://dx.doi.org/10.1253/circj.CJ-16-1286.
[102] C.M. Findley, R.G. Mitchell, B.D. Duscha, B.H. Annex, C.D. Kontos, Plasma levels of soluble Tie2 and vascular endothelial growth factor distinguish critical limb ischemia from intermittent claudication in patients with peripheral arterial disease, J. Am. Coll. Cardiol. 52 (2008) 387–393, http://dx.doi.org/10.1016/j.jacc.2008.02.045.
[103] R.V. Milani, C.J. Lavie, The role of exercise training in peripheral arterial disease, Vasc. Med. 12 (2007) 351–358, http://dx.doi.org/10.1177/1358863×07083177.
[104] N.M. Hamburg, G.J. Balady, Exercise rehabilitation in peripheral artery disease: functional impact and mechanisms of benefits, Circulation 123 (2011) 87–97, http://dx.doi.org/10.1161/CIRCULATIONAHA.109.881888.Exercise.
[105] S.H. Schirmer, D.N. Millenaar, C. Werner, L. Schuh, A. Degen, S.I. Bettink, P. Lipp, N. Van Rooijen, T. Meyer, M. Böhm, U. Laufs, Exercise promotes collateral artery growth mediated by monocytic nitric oxide, Arterioscler. Thromb. Vasc. Biol. 35 (2015) 1862–1871, http://dx.doi.org/10.1161/ATVBAHA.115.305806.
[106] S. Granados-Principal, Y. Liu, M.L. Guevara, E. Blanco, D.S. Choi, W. Qian, T. Patel, A.A. Rodriguez, J. Cusimano, H.L. Weiss, H. Zhao, M.D. Landis, B. Dave, S.S. Gross, J.C. Chang, Inhibition of iNOS as a novel effective targeted therapy against triple-negative breast cancer, Breast Cancer Res. 17 (2015) 25, http://dx.doi.org/10.1186/s13058-015-0527-x.
[107] A.D. Hauer, G.H.M. Van Puijvelde, N. Peterse, P. De Vos, V. Van Weel, E.J.A. Van Wanrooij, E.A.L. Biessen, P.H.A. Quax, A.G. Niethammer, R.A. Reisfeld, T.J.C. Van Berkel, J. Kuiper, Vaccination against VEGFR2 attenuates initiation and progression of atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 27 (2007) 2050–2057, http://dx.doi.org/10.1161/ATVBAHA.107.143743.
[108] C. Yao, J.B. Behring, D. Shao, A.L. Sverdlov, S.A. Whelan, A. Elezaby, X. Yin, D.A. Siwik, F. Seta, C.E. Costello, R.A. Cohen, R. Matsui, W.S. Colucci, M.E. McComb, M.M. Bachschmid, Overexpression of catalase diminishes oxidative cysteine modifications of cardiac proteins, PLoS One 10 (2015) e0144025, http://dx.doi.org/10.1371/journal.pone.0144025.